The South African Journal
of
Medical Laboratory Technology

ORGAN OF THE SOCIETY OF MEDICAL LABORATORY
TECHNOLOGISTS OF SOUTH AFRICA

Vol. 4. No. 2. A QUARTERLY June, 1958

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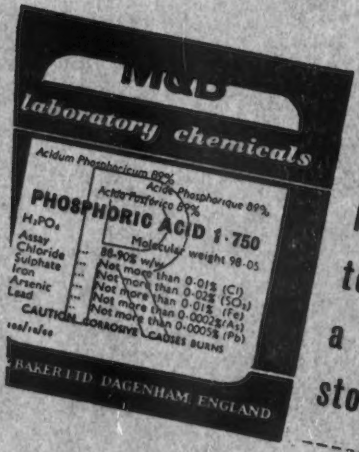

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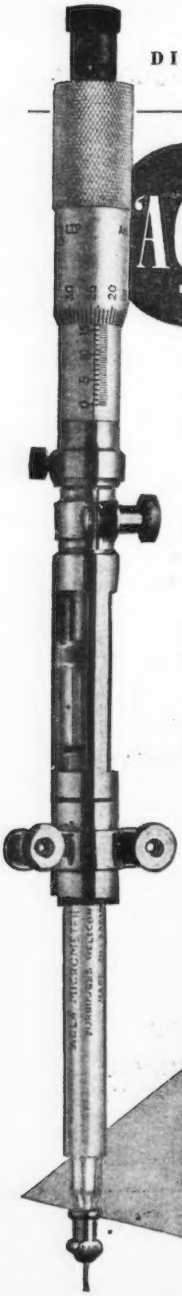

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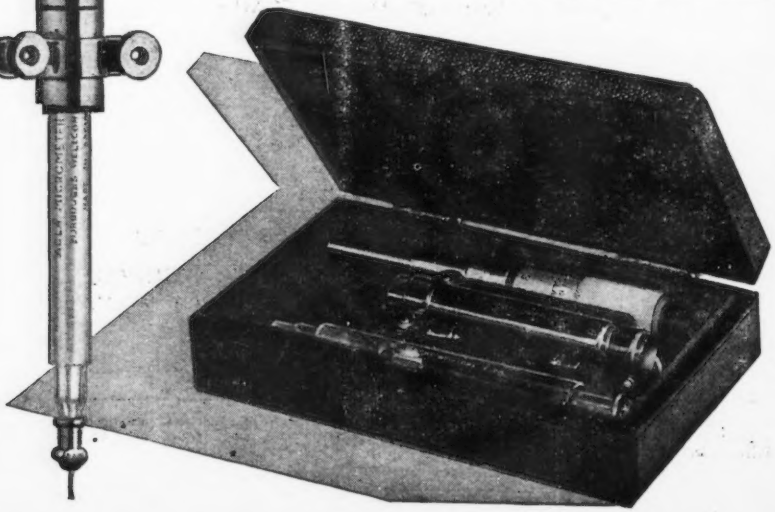

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CONTENTS

REDAKSIONEEL

Die toenemende neiging onder tegnoloë om goewermentsdiens te verlaat vir beter betalende poste, voorspel 'n kleurlose toekoms vir die beroep Mediese Laboratorium Tegnologie.

Die gevolge hiervan kan tweeërlei wees. Eerstens, deur die verlies van opgeleide personeel sal die opleidingsstandaard vir studente en junior tegnoloë ongelukkig affekteer word. Tweedens, en moontlik van ernstiger aard is die onstabiele gevolge sover dit die beroep betref.

In meeste laboratoriums word gehoor :

„Wel ek sal aanbly tot na my eksamens" ; of
„Het jy gehoor H——— is bevorder ?"

In die geval van „toewyding"—wat 'n seldsaamheid is—is dit of ware toewyding, of omdat die persoon besef dat hy nie in die handelswêreld sukses sal behaal nie.

Sekuriteit aan die anderkant behoort ook in aanmerking geneem te word. Ons verstaan dat poste verseker is ten spyte van finansiële agteruitgang. Dit mag so wees, maar dit mag ook wees dat salarisse uitgekort ten tye van geldelike agteruitgang.

Die owerhede moet begryp dat indien hulle die dienste van hulle opgeleide personeel wel behou, hulle salarisse op dieselfde skaal as besigheidsfirmas moet aanbied.

Die meeste tegnoloë in goewermentsdiens verkies om aan te bly, maar as die owerhede nie 'n konkrete oplossing vind vir die salaris probleem wat moet tred hou met die huidige stygende lewenskoste nie, sal die neiging om uit te kyk na ander bronne van inkomste voortgroei by die Mediese Laboratorium Tegnoloog.

EDITORIAL

The growing trend for trained technologists to leave governmental and institutional employment for more remunerative posts in commercial fields would appear to bode ill for the future of the profession of Medical Laboratory Technology.

The results of these moves will have two major effects :—

1. The loss of trained personnel is bound to have an adverse effect on the standards of training of students and juniors.
2. The second is possibly the more serious—the unstabilising effect upon the profession as a whole.

To be heard in most laboratories—

"Well I shall stay until I take my exams" ; or
"Did you hear H——— has got a raise and a car?"

There is the question of "dedication"; those people who expound such are either really dedicated (very few and far between) or know that they are unlikely to make a success of any commercial venture.

The other side of the question must also be considered—security. We are told that posts are secure—regardless of recession—that may be so—so may the possibility of salaries being cut in the event of recession.

It must be realised by the authorities that in order to retain the services of their trained staff they must offer salaries on a similar scale to that of commercial firms.

Most technologists employed in institutional and governmental service would prefer to remain, but if the authorities continue to fail to give any concrete solution to the problem of salaries commensurate with present rising cost of living, there may be increasing tendency to look around to other fields for the M.L.T.

In Memoriam

The late FRANK H. JOSEPH

Mr. Joseph was born in London, 1878, in a house adjacent to the University College Hospital (where his father was Secretary). At the very early age of 16 he commenced his training in the laboratory at Guys Hospital where he continued to work until 1903 when he sailed for South Africa to join Dr. Pakes at the Government Laboratory in Pretoria. This laboratory was subsequently transferred to the tin-shanty building which stood in the present grounds of the S.A.I.M.R., where he worked until the Institute was built, when he was seconded to this staff in 1916.

He left Johannesburg in 1919 to try his hand at farming in Natal but the call to the laboratory was ever strong and so he joined Dr. Burton Nicol's laboratory later in the same year.

In 1931 he joined Dr. Gordon Johnstone at the Addington Hospital laboratory and here he worked until his retirement in 1953.

In his retirement to Pinelands, Cape Town, he still remained active and had many interests such as his beloved bowls and working for charity through the Toc H, which brought him many contacts. He was very keen on charity work for children's institutions and busied himself making and mending children's toys as well as organising "Carols by Candle-light" for the young at Christmas time.

When the S.A.I.M.R. decided on its first training scheme for Medical Technologists in 1917, I was fortunate to be chosen as one of the first four for this course. It was then that I first met Mr. Joseph and soon learnt to appreciate his thoroughness and what a systematic and expeditious worker he was. It was from his lectures in bacteriology that I first came to grips with this subject which was to remain my first love in the Sciences.

When Mr. Joseph later left us in 1919 to go farming and later to join Dr. Burton Nicol I always made a point of calling on him whenever I visited Durban and never for all the many years I knew him did either of us fail to send each other Christmas greetings.

To those who knew him at Addington Hospital—and there are many colleagues still in active practice—Frank Joseph always symbolised the conscientious and thorough worker, even at the advanced age of 75 when he was still active and on his toes.

His kindness and consideration brought pleasure to all who came in contact with him and his modesty and gentleness will remain as an endearing memory to all who knew him.

PAUL ROUX,
S.A.I.M.R., Johannesburg.

LABORATORY TECHNIQUES IN DIAGNOSTIC VIROLOGY

By G. S. TURNER

(University of Cape Town, Medical School)

I.—INTRODUCTION

During the past ten years or so, certainly since the antibiotics came into wide general use, the laboratory aspects of bacteriology have undergone vast changes. The emphasis changes and it is to a group of much smaller infectious agents that the erstwhile bacteriologist must sooner or later turn his attention. It now becomes possible for that not infrequent admission of laboratory failure, "probably a virus infection," to be put to laboratory proof. It is the belief of many that routine medical laboratories will be undertaking diagnostic virus studies in the not too distant future.

Virology is not a new science, the filterable virus era was ushered in some sixty years ago. The techniques have, however, remained principally in the hands of research workers and have not had wide application in the diagnostic laboratory. Certain basic principles are common to both the disciplines of bacteriology and virology. In both are required the recovery and identification of the infectious agent and/or the demonstration of an antibody response related in time to the infection. Aseptic techniques are required, although these can receive considerable assistance from the antibiotics to which most viruses are insusceptible. There is the same reliance on fundamental immunological methods.

On the other hand, in virology there are no simple and direct staining methods, like Gram's stain, which yield immediate information about the identity of a virus. No artificial culture medium has yet been found which will permit virus growth. Without exception, the viruses multiply only in susceptible living cells, and it is by the indirect observation of their effects on such cells that viruses are identified. For a long period such observations were made on the intact susceptible laboratory hosts, virus infected material being inoculated from one animal to another. Since the host range of different viruses varies widely (and is itself an aid to identification), this entailed the maintenance of large stocks of a wide variety of animals, *e.g.*, monkeys for poliomyelitis, ferrets for influenza, together with the more common laboratory rodents.

Levaditi (1906) considered Borrel to have first used chick embryos for the study of infectious agents. Rous and Murphy (1911) used embryonated eggs in their studies on a transmissible fowl sarcoma. Goodpasture (1933) gave great impetus to virology in drawing attention to the chick embryo as a suitable vehicle for the propagation of viruses.

From 1933 onwards some 30 varieties of viruses or rickettsiae were cultivated in embryonated eggs. Among them may be mentioned the mumps, Newcastle disease, influenza group viruses, all of which grow well and are relatively easily characterised. Yellow fever, vaccinia, rabies and several encephalitis viruses also grow well in eggs. In many, if not all cases, adaptation to growth in chick embryos results in a change in characteristics, and several egg attenuated strains of virus have been used in vaccine production. A whole series of chick embryo methods have grown up. Different sites of inoculation, exposing different tissues and cell types to the viral agents, have been used. The site in the embryo in which a virus most actively reproduces often gives information valuable in identification. Rapidly proliferating cells eminently suited to virus growth are also found in immature animals and Dalldorf and Sickles (1948) used suckling mice for the isolation of new viruses now known as the Coxsakie group. Their techniques with unweaned mice have been successfully used with many other viral strains.

An *in vitro* source of living cells has occupied the attention of virologists for many years. Harrison (1907) developed the first comparatively simple method of tissue culture. Steinhardt, Israeli and Lambert (1913) showed that vaccine virus remained viable in fragments of rabbit cornea surviving in plasma drops. Twelve years later, Parker and Nye (1925) demonstrated the active multiplication of vaccine virus in rabbit testis cultivated in plasma. Since that time many of the viruses and rikettsiae have been cultivated in serial passage in tissue culture.

Gey (1933) was largely responsible for developing roller tube techniques for the massive culture of tissues. Tissue fragments were embedded in a plasma coagulum and alternately bathed in nutrient and exposed to the amosphere within the vessel. Using such techniques Robbins, Enders and Weller (1950) succeeded in cultivating all the known types of poliomyelitis virus and made possible the development of a vaccine. An immense simplification in procedure has followed this achievement—cells dispersed from minced tissue by the action of trypsin can be grown on solid surfaces in direct contact with the nutrient ; the floors or walls of glass vessels can be relatively uniformly populated with standard cell types and the complex nutrients have given way to easily prepared serum-peptone-buffer mixtures. Such cell sheets, when infected with virus containing material, can be easily examined microscopically for characteristic cytopathogenic changes. They can be used in serum neutralisation tests and, in fact, for almost all the purposes for which animal and chick embryo techniques have hitherto been used. It cannot be said that the latter techniques have been completely superseded, but the fact that tissue cultures can be handled and observed in the laboratory much in the same way as ordinary bacteriological culture media, has immense advantages. There are methods, Dulbecco (1952), Cooper (1955), Hsiung and Melnick (1957), which permit the plating of infected material in a manner much akin to that used in plating bacteriological specimens.

Hanks (1955) says that much of what is called tissue culture should more correctly be termed cell culture. Complex tissues may be used as starting materials, but on culturing under conditions which ensure maximum growth, only particular cell types survive and grow, and no organisation into tissues occurs. To this end, single cell strains, notably the Hela strain of cancer cells, have been developed for virological use. Derived originally from a cervical epidermoid carcinoma by Scherer, Syverton and Gey (1953), they may be maintained in serial subculture in suitable medium in a fashion very similar to the maintenance of a culture of yeast cells. The Hela strain of cells is susceptible to a wide variety of viruses and the relative simplicity of their maintenance has led to their commercial production in America for virus studies.

It is with the development of these new tools and the continued use of the old that the virologist may now make a much greater contribution to the diagnosis of virus infections. The application of these new methods has already borne fruit in the virus diagnostic unit set up here. It has given valuable assistance in the diagnosis of poliomyelitis, two outbreaks of Coxsakie disease have been investigated with full laboratory co-operation in a manner analagous to that of a typhoid epidemic, Kipps *et al* (1958) and many completely new virus strains have been isolated.

It might be said that the virologist now finds himself in a position comparable with that of the bacteriologist in the Pasteur-Koch-Erlich era of bacteriology. Difficulties are inherent in the introduction of any new technique and its application as a general laboratory method. It is with this in mind that the present series of articles has been contemplated. In ensuing articles the writer and his colleagues will give details of the methods which are in use here. It should be emphasised that such methods will seldom be original. It is hoped, however, that collection "under one roof" as a series of techniques may be of value.

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DEGENERATIVE HEART DISEASE

Can its development be hindered by alterations in eating and other habits

By A. R. P. WALKER, M.SC., PH.D. and I. BERSOHN, B.SC., M.B., CH.B.

Dr. Walker is head of Human Biochemistry Research Unit of the Council for Scientific and Industrial Research and the South African Institute for Medical Research.

Dr. Bersohn is head of the Ernest Oppenheimer Research Unit in Cardio-vascular Disease., South African Institute for Medical Research.

Among many White populations, including European South Africans, degenerative heart disease is the leading cause of mortality after 45 years of age, and is responsible for four out of ten deaths of adults. Evidence indicates that during the present century the incidence of the disease has increased markedly, and the mortality rate is still going up.

Briefly, from the time of youth in men, and later, in women, arteries suffer from certain well defined morbid processes whereby the character of the walls is affected ; furthermore, changes may be induced in the tissues which they normally supply with blood. Deposits of fatty material and calcium salts, known as atheroma, often lead to ulceration and the development of plaques, sometimes of stony hardness. This atherosclerotic process is thus a variety of arterial degeneration, which is confined very largely to the inner lining of the vessel walls. Apart from the elasticity of the blood vessel walls being deleteriously affected, a narrowing of the lumen, or partial occlusion, occurs. When these changes take place in the coronary arteries which nourish the heart itself, the development of angina pectoris is favoured, this occurring when there is a spasmodic decrease in the blood supply to the heart muscle or some part of it. Should total occlusion occur, either from progressive narrowing or from blocking by a blood clot (thrombus), the person may die instantly from coronary thrombosis ; or, if the person recovers, the occlusion will cause an infarction or death of that portion of the heart muscle supplied by the occluded branch of the coronary tree, with subsequent replacement by fibrotic or scar tissue. A person may have one, two, but very seldom more than three attacks. The tragedy is that a first and often fatal attack may take place in outwardly healthy people who may be thus struck down without warning. At one time, it was thought that these degenerative changes of the blood vessel walls were an inevitable accompaniment to age ; but to-day, the concensus of opinion is that the degeneration is a disease process, which may be prevented, or at least retarded. It will therefore be obvious that much of the present-day research is directed into endeavouring to detect the

earliest possible changes, clinical, biochemical, haematological, etc., which herald or accompany the development of degenerative heart disease. What is the cause of this disease ?

Many factors, of course, are implicated, over some of which we have no control, but over others we have a measure of control. In the first category there is the congenital factor whereby some individuals are born with or develop blood fat levels (believed to be of considerable relevance in the development of atherosclerosis) higher than are conditioned by diet. Next, there is the sex factor, for until well after the change of life women are far less prone to coronary artery disease than men. Notwithstanding, it must be pointed out that in Bantu men the disease still occurs less frequently than in White women. Thirdly, there is the factor of age, for atherosclerosis is essentialy a progressive disease. Against these, which one might call imponderable factors, one can obviously do nothing. The other factors implicated are of environmental type, and include diet, stress, exercise, and smoking.

DIET

It is generally believed that of these environmental factors, diet is probably the most important. Furthermore, evidence indicates that of the various components of the diet, fat is implicated the most. Briefly it has been thought, at least until recently, that the more fatty foods one eats the more is one liable, directly or indirectly, to develop degenerative heart disease—and vice versa. While this generalisation may apply to Western populations, it is not true for all populations. Thus, the Eskimos and the inhabitants of the Yukon region consume a diet very high in animal and marine fat, yet apparently those people suffer very little from degenerative heart disease. Again, the Yemenite Jews, although used to a high intake of vegetable fat, likewise appear to suffer little in this respect. More recently, it has been shown, not only by groups of American workers, but also by the C.S.I.R. Clinical Nutrition Unit of the University of Cape Town, of whicn Professor J. F. Brock is head, that the blood fat picture is affected not solely by the quantity of fat ingested, but by the type of fat consumed. Briefly, speaking generally, fats of vegetable and marine origin (mainly liquid at room temperature) have a lower capacity to elevate blood fat levels compared with fats of animal origin, which are usually solid at room temperature. The former fats have a relatively high proportion of unsaturated fatty acid carbon chains and have what is called high iodine number ; animal fats are more saturated, having lower iodine numbers. Vegetable fats, which have been hydrogenated to make them solid, change their character, their iodine number becoming reduced and their capacity to elevate blood fat levels being increased. In this respect, evidence is accumulating that population groups characterized by having low blood fat levels (in particular, low serum cholesterol levels) have a relatively low incidence of coronary artery disease.

In South Africa research workers are particularly favoured in living side by side with a primitive population among whom the incidence of atheroma and coronary artery disease is only about a tenth of that of the local White population. This quantitative difference (qualitatively it has been known for some time) was found in the course of a comprehensive study on cadavers by pathologists from the South African Institute for Medical Research, where a number of other relevant contributions has been made. Thus, it has been found that blood serum cholesterol levels are lower, and the rise with age minimal, in Bantu compared with European adults. At birth, levels have been found to be virtually identical for babies of both races. It has also been found that the serum lipo-protein (fat) picture in the Bantu, even of the elderly, resembles the picture found in young Europeans. Again, from the chemical analysis of a large series of aortae (the main artery from the heart) from Bantu and Europeans of different age groups, it has been found that, in general ,aortae of the Bantu do not become older than those of young Europeans. Several other studies are in progress or are planned for the future to learn why the blood vessels of the Bantu, speaking relatively, retain their perennially youthful character. Apart from endeavouring to throw light on the aetiology of degenerative heart disease, the immediate aims are to define as precisely as possible the relevant difference between the Bantu and European population groups and, further, as noted before, to seek to find means of detecting the earliest changes that accompany the development of atherosclerosis and its complications. The recent establishment of the Ernest Oppenheimer Unit for Research in Cardio-vascular Disease at this Institute will greatly expedite the objectives described. One of the first decisions of the Unit was to send a research worker to universities and research institutions in the United States and Britain to learn of current research and developments in techniques in atherosclerosis, so that the research effort of the Unit may be put to the best advantage.

The aims are, of course, laudable enough. But the question arises, what can be done *now* to arrest or hinder the development of atherosclerotic lesions in the large proportion of adults who presumably are already affected by degenerative heart disease ?

It is heartening to consider that during the last war, when many Western populations ate less fat, the reduction was accompanied by decreases in the mortality rate from degenerative heart disease. Yet, when peace came and the diet reverted to its pre-war pattern, the relevant mortality rate rose even higher than it had been before the war. The position, however, is not quite as straightforward as it sounds, for wartime diets entailed not only a reduction in fat intake but decreases in the consumption of animal protein foods, yet increases in carbohydrate foods, and certain vitamins and mineral salts. It is not altogether justifiable, therefore, to ascribe the changes in mortality rate as being associated solely with alterations in the consumption of fat, without reference to the other concomitant dietary changes.

Assuming that the amount and type of fat consumed, at least in our pattern of diet, is the most influential dietary factor, to what level must the intake fall before worthwhile results are to be expected ? In England, during the war, the fat intake fell by about one-seventh ; the fall in the death rate from coronary heart disease was small but significant, particularly at ages over 50 years. In Norway, however, where the fat intake fell by 40 per cent, the corresponding mortality rate decreased by 25 per cent. In other words, it would seem that in the dietary pattern prevailing, fat intake has to be reduced quite considerably, say, to about 2 oz. per day, before the mortality rate from degenerative heart disease may be expected to fall markedly. Such a diet, of course, must be continued not for months, or even for years, but all the time.

But apart from war-time experiences, it is illuminating to compare the mortality rate from degenerative heart disease in the United States with that in, say, Italy where the general intake of fat is approximately half of that in the former country. The total number of deaths annually from this disease in the United States is about 38,000, but were the mortality rate the same as in Italy, then there would be only 13,000 deaths. It must, of course, be assumed that the large difference in fat intake is the sole or even the most important dietary feature, yet the figures themselves indicate that the position is a long way from being desperate in so far as the possibility of reducing the incidence and mortality rate from degenerative heart disease.

For those who think that their fat intake is excessive and are considering reducing it (and this applies especially to the obese), there are a number of reasons for encouragement. Firstly, a reduction in fat intake is invariably accompanied by a beneficial change in the blood lipo-protein (fat) picture, which, *prima facie*, is a step in the right direction. Next, in patients showing manifestations of the disease, it has been demonstrated that gross reduction in fat intake when accompanied by specific therapy, can cause not only a beneficial change in lipo-protein picture, but also a marked amelioration of symptoms. A third reason, to which quite inadequate attention has been paid, is that a lowered fat intake such as occurred in war-time Britain, Switzerland, and other countries, was accompanied by unequivocal improvements in the death of the total populations. To give just one example. At the end of the war in Britain, it was calculated that 100,000 diabetics were alive who would have been dead had the trends of mortality at the beginning of the war continued. The lives saved were deemed to be due to the reduction in the fat content of the national diet.

NON-DIETARY FACTORS

Race.—Many have wondered whether a racial factor is involved in protecting the Bantu against atherosclerosis. Present evidence indicates that this is unlikely to be the case. For example, the incidence of coronary artery disease has been shown to be just as high in certain Negro groups

in Chicago, as in the White population. Again, while the disease is uncommon in Java, it has been shown to be high in Javanese who have resided in Holland for some time.

Stress.—There is a difference of opinion regarding the extent of the part played by stress in promoting the development of coronary artery disease, and only very long-term observations such as are being carried out in certain cities in the United States, are likely to clarify the situation. It would be wrong to suppose, however, that population groups, or individuals, among whom the severer complications of atherosclerosis are rare, are, *ipso factor*, not over-burdened by stress. It is sometimes considered that the Bantu, with their happy-go-lucky temperament, and their living more in the present than planning for the future, stand somewhat apart from the stressful way of life inherent in White civilization. That may be so in certain respects, but have they not stresses of their own although little recognised by the White population ? To give an example, some time ago one of the writers, in the course of an investigation, desired to determine certain constituents in the stools or faeces of the Bantu employees at the Institute, who were consuming an adequate diet of known composition. Out of 150 boys, and offering 5s. per stool, despite begging and even threatening, only 6 stools were forthcoming—the reason being that it was believed that through the medium of their stools some far-reaching harm could be done to the givers. It is not believed, therefore, that the Bantu suffer little from coronary heart disease merely because they lead less stressful lives than Europeans. It is considered that they have stresses peculiar to themselves, the profoundness of which are little understood by us.

Exercise.—In Britain it has been shown that there is a significantly higher incidence of coronary artery disease among those employed in less active compared with the more active occupations. To what extent exercise retards the development of atherosclerosis is not known. Nevertheless, recently Dr. Paul White, the eminent cardiologist attending President Eisenhower after his attack of coronary thrombosis, is a firm believer that exercise is implicated, and he has been earnestly counselling us to buy bicycles. Progressively increasing industrialization and urbanization have, of course, made great changes in the activity of man's occupation. Thus, in South Africa, in 1891, only about one-third of the total White population dwelt in urban areas ; by 1941, the proportion was over two-thirds, and it is still increasing. Again, in the United States, several generations ago, out of every 20 men, 19 were employed directly or indirectly, in agriculture ; but to-day, with the change over from an agricultural frontier civilization to an industrial urban civiliaztion, the above ratio has been almost reversed. Literature, of course, abounds with instances of people who have looked askance at the changing way of life and its various ramifications, including decreasing exercise. Thus, Sir William MacEwan, Regius Professor of Surgery at the University of Glasgow, in his Huxley lecture in 1904, said, "From

the way prevalent in many places, man seems to act as if food should be thrown into the stomach, as a sandwich into a pocket and the lid closed." This, MacEwan contrasted with, "Primitive man had to search for his food, and sometimes he had a hard physical fight to get it. When he found it, as he had been looking forward to it, he was fit for it and enjoyed it." On much the same note, and that long before MacEwen's time, John Dryden rhymed, "Better to hunt in the fields for health unbought, than fee the doctor for a nauseous draught." The popular tradition of the "healthy savage," like that of "the good old days," must, of course, be treated with some reserve. Nevertheless, it is certain that, accompanying decreased activity, degenerative heart disease is commoner now than it was a few generations ago ; that at present the disease is more common among the less active section of the White population ; further, that the disease is relatively rare among the pigmented populations of the world, who earn their living with their hands rather than with their heads. Our own impression is that, in so far as activity is significantly implicated in the present issue, it is not so much activity *per se*, but rather that way of life and all that is bound up with it which attaches to population groups whose habitual occupation demands more than a modicum of exercise. Assuming the latter factor is of some relevance —what is one to do ? It is suggested that those of us, whether young or old, whose activity is limited almost to our running to catch a bus, might well derive benefit in more ways than one by following Dr. Paul White's advice and indeed buy a bicycle !

Smoking.—In the United States, Dr. E. C. Hammond and Dr. D. Horn have recently made a profound study of the relationship between human smoking habits and death rates in 187,766 men. Amongst other observations, they found that disease of the coronary arteries was the primary cause of death in 45·6 per cent of cases. They found that in the age group 50–64, the death rate of those who smoked a pack or more of cigarettes (20 a day), was more than *twice* as high as those who never smoked. Here, again, one must be cautious over attaching undue significance to smoking *per se*, but rather should account be taken of the total picture of nutritional habits, mental make-up and so forth, which is associated with heavy smoking individuals. However, it cannot be maintained that lack of smoking among the Bantu is partly responsible for protecting these people from atherosclerosis. For the smoking habit among them is common enough ; moreover, cancer of the lung appears to be equally common among Bantu and European adults, according to the comprehensive cancer survey being undertaken by Dr. J. Higginson and Dr. A. G. Oettle of this Institute.

The title of this article posed the question : can the development of degenerative heart disease be hindered by alterations in eating and other habits ? We certainly wish we could give a categorical affirmative answer. We think that the answer is "Yes." Perhaps the mainstay of our hope is the important wartime observation already mentioned,

namely, that in certain European countries the death rate from the disease did, incontestably, go down ; and we more than suspect that the chief influencing factor was the change in diet, in particular, the change in the amount and type of fat consumed. We also regard of importance certain relevant investigations, such as that of Dr. John Gofman of the University of California, who found that patients who have had an attack of coronary thrombosis and who subsequently greatly reduced their fat intake, had a lowere incidence of further attacks compared with patients who did not reduce their fat intake. Summarizing, then, our counsel would be : to the obese, reduce weight (numerous benefits will follow) ; to the adult of normal weight, reduce the intake of fat, at least of animal origin (and don't be afraid to make up the deficit of calories with brown bread and jam) ; to those under stress, relax ! (We would like to know how to do just that ourselves !) ; to the lazy, take more exercise (for example, get rid of the garden boy) ; to the heavy smokers, smoke less. In other words, our counsel reduces to one of moderation, a counsel as old as time itself ; indeed, we are reminded of a quotation from Plutarch : "There are two sentences inscribed upon the Delphic Oracle, hugely accommodated to the usages of man's life : 'Know thyself' and 'Nothing in excess'."

It would be idle to deny that our knowledge of the cause, diagnosis, and treatment of degenerative heart disease is woefully inadequate. Nevertheless, as noted, there are many grounds for hope, and moreover, there is probably no other health problem now being as carefully and extensively investigated in research institutions and universities in Johannesburg, Cape Town, Pretoria and elsewhere in Africa, as well as throughout the world.

(*This article originally appeared in* LANTERN *and is reprinted by kind permission of the Editor.*)

THE HUMAN ISOSPORA

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The genus *Isospora* includes a number of gut parasites which are fairly common in domestic animals, but which, until recently, were considered to be rare in man. There has been considerable doubt as to whether the representatives of this genus found in man were, in fact, true human parasites ; that is to say, whether they were capable of undergoing any part of their life history within the human body, or whether the cysts were ingested and passed through the gut unchanged. Since, in the King Edward laboratory, we have recently been finding a surprisingly large number of cases of Isospora infection during routine examination of stool preparations after concentration by the zinc sulphate

flotation method, it may be of interest to review some of the observations made in this laboratory, and by other investigators, about these parasites.

From the classificatory point of view, the Isospora are placed in the *phylum Protozoa*, which includes all unicellular animals ; and in the class *Sporozoa*, which comprises a number of parasites with no permanent locomotory organs, most of their life being spent in an immobile state. The members of this class have characteristic life histories, and, as their name implies, they produce spores. Sub-division of the class places the *Isospora* in the order *Coccidia*, which includes a number of parasites affecting the epithelial cells of the gut.

The life cycle of the *Isospora* is presumed to approximate to the following pattern : when a spore penetrates an epithelial cell of the gut of the host, it grows into a large, round body and divides to form a cluster of merozoites. The cell is ruptured, and the merozoites are released to infect new cells and repeat the cycle. This is the asexual cycle. The sexual cycle occurs when a merozoite, on invading a cell, becomes differentiated into either a male or a female gamont. The male gamont divides to form a number of biflagellate male gametes, while a single female gamete is formed after extrusion of some nuclear material from the female gamont. The epithelial cells now rupture, and the gametes unite to form a zygote, which is a round body containing a central granular mass. The zygote develops a protective wall and is now ready to be passed out in the stool as an oocyst. The granular mass contained in the oocyst is known as the sporoblast. This divides into two, and, from the granular material in each sporoblast, four sporozoites are formed. The residual granular material is known as the residual body. At this stage, the sporoblasts are known as sporocysts, and the parasite is now assumed to be ready to infect a new host. This is brought about by ingestion of the oocysts. The sporocysts are now released from the oocyst wall, and the sporozoites or spores are in turn extruded from the sporocysts to penetrate the gut epithelial cells and start the cycle anew.

Most of this life cycle has never been demonstrated in *Isospora*, and it is assumed by analogy with *Eimeria*, which is a related *Coccidian* parasite, differing from *Isospora* in that there are four sporocysts, each containing two sporozoites, as opposed to two sporocysts, each with four sporozoites, as is the case in *Isospora*.

The genus *Isospora* was first founded by Schneider in 1881, with *Isospora rara* in the black slug. Other species have since been found in frogs, birds, dogs, cats, pigs and foxes, so the genus is obviously fairly widespread in distribution. The first human species of *Isospora* was described in 1860 by Virchow, who found the sporocysts in the villi of the small intestine. This parasite was later named *Isospora hominis*, by Raillet and Lucet in 1901. Mature sporocysts have since been reported from stools on a limited number of occasions.

The next species of human *Isospora* was discovered in 1915 in the stools of patients invalided from Gallipoli. Wenyon named this parasite *Isospora belli*, meaning *Isospora* of the war. In this species, the immature oocyst, as opposed to the sporocyst, is passed in the stool. At this stage, it may contain either one or two sporoblasts, which, given oxygen, will mature into sporocysts in about two days at room temperatemperature. The oocyst is generally oval in shape, about 25μ long, and about twice as long as it is broad. It is very light, and, in microscopical preparations, will float above the plane of focus of the rest of the material on the slide. This may explain why it has not been found by several investigators. *I. belli* is generally described in textbooks under the name of *I. hominis*, since the form found in stools is considered to be the oocyst of the same species as that described by Virchow. Observations in this laboratory suggest, however, that the two types are, in fact, different species.

In the first place, out of 78 recent cases of *Isospora* infection found in this laboratory, only four have been mixed infections of *I. hominis* and *I. belli*. This proportion would be expected to be higher if the two types were the same species. Furthermore, in these mixed infections, there were no intermediate stages between the immature oocyst of *I. belli* and the mature sporocyst of *I. hominis*.

There are also structural differences between the sporocysts of the two species, in that the sporozoites of *I. belli* are relatively larger than those of *I. hominis* and tend to fill up more of the space inside the sporocyst wall. Furthermore, the residual body *I. belli* tends to be more central in position than does that of *I. hominis*.

The third species of human *Isospora* is *Isospora natalensis*, named by Elsdon-Dew in 1953, of which there have been only two recorded cases, both from King Edward Hospital. The oocyst of this species differs from that of *I. belli* in that it is considerably more spherical, being about 27μ long by about 23μ wide. Also, the sporoblasts take up an equatorial position in the oocyst, as opposed to the polar position in *I. belli*. With regard to the position of the sporoblasts, *I. natalensis* is similar to *Isospora rivolta*, which is a parasite of cats, but differs from this species in two known respects. Firstly, the sporulation period is different, as in *I. natalensis* the sporozoites develop within 24 hours of the passing of the immature oocyst, while in *I. rivolta* the sporulation time is 96 hours; and secondly, the oocyst of *I. natalensis* appears to be significantly larger than that of *I. rivolta*, which measures about 22μ by 17μ. Of these two factors, the size difference is probably the more significant one, since it has been noted that the sporulations time of the species *I. bigemina* is different in the fox from that in the dog and the cat.

The question now arises as to whether the human *Isospora* may be considered to be true human parasites or not ; that is to say, whether they undergo various stages of their life cycle within the human body.

The life cycle, the historical background and the diagnostic characteristics of the *Isospora* species found in man have now been considered.

The question now arises as to whether the human *Isospora* may be considered to be true parasites or not ; that is to say, whether they undergo various stages of their life cycle within the human body. On this basis, the question as to whether man is the natural host for these parasites is only one of secondary importance. It may be pointed out, however, that *I. belli* is morphologically distinct from the *Coccidian* species which have yet been found in other animals, and attempts to infect cats with *I. belli* have so far been unsuccessful. A certain resemblance between *I. hominis* and *I. bigemina*, which is found in some domestic animals, has been noted. Cross-infection experiments must obviously be carried out in this connection before any conclusion is reached.

We have several indications for supposing that the human *Isospora* may be regarded as undergoing a course of development within the human body.

In the first place, *Isospora* infection seems to be much more widespread than has previously been supposed. In the last nine months we have isolated 78 cases of *Isospora* infection, representing an incidence

of about 1 per cent of *I. hominis* and about 0·2 per cent of *I. belli* in the total population studied. In children, however, the incidence of *I. belli* is about 2·5 per cent. These figures are probably a conservative estimate, since it is likely that many cases of *Isospora* infection may have been overlooked, especially, perhaps, of *I. hominis*, where the sporocysts are small, often very scanty, practically transparent, and, like the oocysts of *I. belli*, tend to float above the plane of focus of the rest of the material on the slide.

Although, in many cases, the *Isospora* individuals in a single preparation were scanty in number, cases were observed in which up to 800 sporocysts of *I. hominis*, up to 2,000 oocysts of *I. belli* or up to several hundred oocysts of *I. natalensis* were detected under one coverslip. In the case of *I. belli*, unusually large numbers of occysts were often observed in children's stools. This agrees with the observation that in domestic animals coccidiosis is largely a disease of the young.

In the great majority of cases of *Isospora* infection we have been able to recover the parasite from the patient's stools on occasions subsequent to the initial isolation. Oocysts of *I. belli* have been recovered from patients' daily stool specimens for periods of up to 27 days, sporocysts of *I. hominis* have similarly been observed daily for periods of up to three weeks, while oocysts of *I. natalensis* were recovered in large numbers from a patient for four days. Thus, on the basis of figures alone, it seems that it is practically conclusive that the *Isospora* are human parasites.

Further evidence in support of this postulate lies in reports of *Isospora* from the human gut epithelium. It has already been mentioned that Virchow first reported *I. hominis* from the villi of the small intestine. At least two other investigators have since reported finding Coccidia in the human gut, but the species of these animals was unfortunately not determined. We have been able to recover oocysts of *I. belli* from the duodenal contents of a hospitalised patient, who for a week previously had been passing oocysts in large numbers in his stools. Attempts, however, to recover *Isospora* from post-mortem duodenal scrapings from children in this hospital have so far been unsuccessful, but we have not yet been able to investigate in this manner any patients who were known to be suffering from an *Isospora* infection.

Attempts have been made to hatch out the oocysts of *I. belli* and the sporocysts of *I. hominis* by placing these in human gastric juice and then transferring them to duodenal juice. Here, again, this laboratory has so far met with no success, but this may be due to the fact that we have been experimenting with oocysts and sporocysts which had previously been concentrated in zinc sulphate. This chemical may possibly exert an inhibiting effect on enzyme systems operating on the parasite, so we are at present investigating alternative methods of cyst

concentration. Parallel experiments conducted along these lines by two German investigators, Herrlich and Liebmann, where zinc sulphate was not used as a concentrating medium, have in fact, resulted in the rupture of the cyst walls and the liberation of the sporozoites. No movement, however, was observed in these bodies, so free sporozoites which are undoubtedly still alive have yet to be observed.

Herrlich and Liebmann have reported a case which, again, strongly supports the postulate that the human *Isospora* are true human parasites. After the initial isolation of oocysts of *I. belli* in a stool, there was a lapse of four days in which the patient produced no faecal evidence of an *Isospora* infection. Then, for three days, bodies which were later shown to be the immature zygotes of *I. belli* were liberated. After another lapse of two days, oocysts were again observed in the stools for two days. We have here twice observed persistent secretion of rounded, granular bodies in connection with heavy infectionsof *I. hominis*, and have suspected these bodies to be the immature zygotes, but, unlike Herrlich and Liebmann in the case of *I. belli*, we were not successful in maturing these to the oocyst stage. This, again, may have been a reflection on our use of zinc sulphate.

In many cases, human infection with *Isospora* spp. seems to be associated with definite dysenteric symptoms. The classic example of this is the case cited by Connal, in which a laboratory worker accidentally ingested material containing ripe oocysts of *I. belli*. After six days he suffered an attack of diarrhoea, which persisted for 28 days. Oocysts were first found in the stool 22 days after the beginning of the diarrhoea and were still being liberated for seven days after the disappearance of the disease. This suggests an association of diarrhoea with the probable schizogony cycle of *Isospora*, and agrees with the coccidiosis syndrome in domestic animals. Herrlich and Liebmann, in the investigation of 27 cases of *Isospora* infection in soldiers in North Africa, reported a history of dysentery previous to the isolation of *Isospora* in the majority of cases studied. In most of these cases, no evidence of amoebic or bacillary dysentery could be found.

With regard to the cases of *Isospora* examined in this hospital, the majority of these are complicated by infection with *Entamoeba histolytica*. In a few cases, however, particularly in children, symptoms of dysentery occurred in conjunction with *Isospora* infections in the absence of evidence of bacillary or amoebic dysentery. Although some patients appear to suffer no ill effects from a heavy *Isospora* infection, it seems possible that infection with *Isospora* may sometimes be one of the causes of the gastro-enteritis which is very prevalent among the infants in this hospital.

It is clear that much remains to be found out in connection with the human *Isospora*. The missing stages in the life cycle must be observed, the possible relationship between these parasites and related species

in domestic animals must be studied, the clinical picture must be more clearly elucidated, and the therapeutical effects of various drugs must be determined. Even in domestic animals, where coccidiosis is a recognised and fairly widespread disease, there is a considerable difference of opinion as to the treatment for this condition. The available evidence, however, strongly supports the acceptance of the human *Isospora* as valid human parasites, and recent observations have clearly indicated that a further study of these parasites may represent a profitable matter for investigation.

BIOCHEMISTRY ABSTRACTS

A titration method for the determination of calcium in serum using a new indicator. Andersch, M. A. *Journ. Lab. Clin. Med.*, 1957 : **49/3** (486–489).

An indicator ("calcein") specific for calcium in the presence of magnesium is used for the titration of serum edetic acid calcium with pH. 12. The indicator is prepared by condensing iminodiacetic acid with fluorescein, but has not been isolated as a pure product.

The determination of phosphorus and phosphatase with N-phenyl-p-phenylenediamine. Dryer, R. L., Tammes, A. R., Routh, J. I., *Journ. Biol. Chem.*, 1957 : **225/1** (177–183).

A reagent for the reduction of phosphomolybdate which is stable and fast and which contributes to the optical absorbance of the final solution, has been proposed. The maximal absorbance is obtained in ten minutes or less after addition of the reagent and is constant for 1.5 hours. An absorbance maximum from 340 to 385 millimicrons has been observed.

Determination of serum and tissue cholesterol. Herrmann, R. G. *Proc. Soc. Exp. Biol.* (*N.Y.*), 1957 : **94/3** (503–505).

A simple and sensitive method for determination of serum and tissue total cholosterol is described.

An improved type of apparatus for manometric microdetermination of CO_2 in small volumes of plasma or serum. Rappaport, F., Eichhorn, F., Nutman, M. *Clin. Chim. Acta*, 1956 : **1/4** (305–310).

Description and instructions for operation of an improved form of micro-Van Slyke apparatus, suitable for the determination of CO_2 in 0·05 to 0·1 ml. plasma.

A simple microcolorimetric method for the determination of pyruvate in 0·1ml. samples of blood. Rindi F., Ferrari, G. *Experientia* (*Basel*), 1956 : **12/10** (398–399).

The procedure is a micro adaptation of the Friedemann and Haugen colorimetric method. (*Journ. Biol. Chem.*, 1943) (2 : 4 dinitrophenylhydrazine ; toluene extraction.) This adaptation gives results which are only slightly lower than those of the basic macro method and requires only routine apparatus and supplies. A.J.S.G.

EXAMINATION RESULTS

The following members successfully completed the course of study (Johannesburg) in 1957 :—

M. Bleloch (Miss).
M. Bromfield (Miss).
M. Dowell (Miss).
N. Divaris (Mr.).
P. Johnson (Mr.).
M. Salter (Miss).
A. Sacks (Miss).
J. Woods (Miss).

Diplomas were presented to the above candidates by Lady Lister at a gathering held at the South African Institute for Medical Research in April, 1958.

Miss Averille Sacks was awarded the Sir Spencer Lister Memorial Prize.

The Sir Spencer Lister Memorial Prize is an award made to the student with the outstanding academic record over the course. It is awarded by the Director and Board of the South African Institute for Medical Research to a local candidate.

INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY

The triennial conference of the Institute will be held at the University of Bristol from 13th to the 20th September, 1958. An interesting programme has been planned and copies of the handbook are available from the General Secretary, 74 New Cavendish Street, Harley Street, London, W.1.

NOTICE TO CONTRIBUTORS

All contributions are to be addressed to:—The Editor, The South African Journal of Medical Laboratory Technology, Room 213, Dept. of Pathology, Medical School, Umbilo Road, Durban.

Contributions may be written in English or Afrikaans, and should preferably be typed in double-spacing on foolscap sheets on one side of the paper only.

Figures should be drawn in Indian ink, and all figures and tables should be labelled as such (e.g. Figure 1, Table 1, etc.).

Authors should make adequate references to previous works on their subjects. These should be set out as follows:—Author's surname and initials of Christian names; the year of publication (in parentheses); the name of the journal, which should be abbreviated according to the World List of Scientific Periodicals (see below); the volume number (underlined); and the first page reference.

Example:—Moron, I. B. (1960). J. unsuccess. Med., 20, 99. References to books should give the author's name and initials, the year of publication, title of book, name of publisher, and town in which published.

References should be arranged in alphabetical order of the authors' surnames. If more than one work by the same author is listed, these should appear in chronological order.

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Title abbreviations according to World List of Scientific periodicals

All nouns commence with capital letters, and adjectives small letters. Articles, conjunctions and prepositions are omitted.

Examples:—

J. Amer. med. Ass.	*S. Afr. J. clin. Sci.*
Lancet	*Stain Tech.*
Amer. J. clin. Path.	*J. Bact.*

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